

10054712.11304

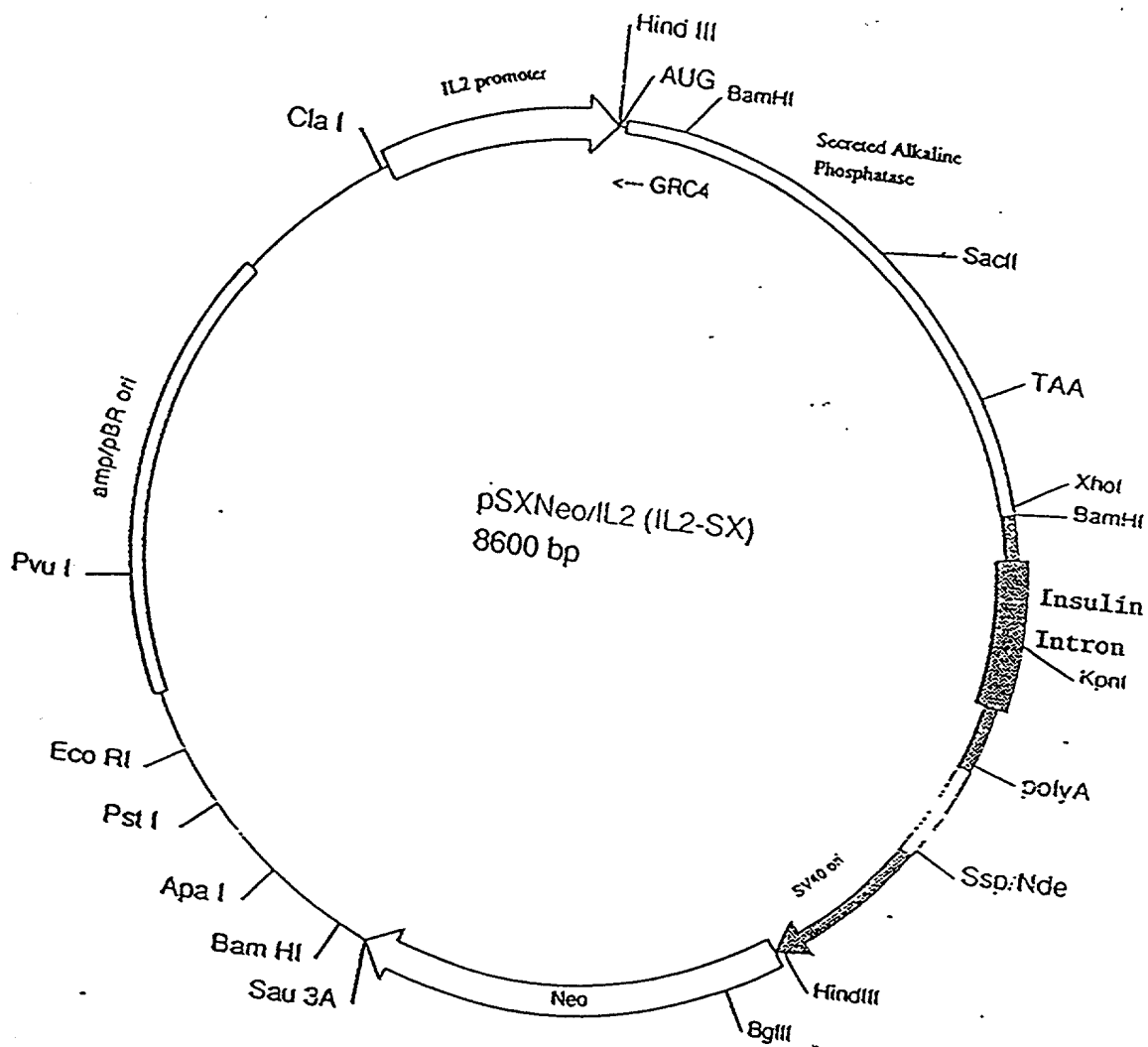
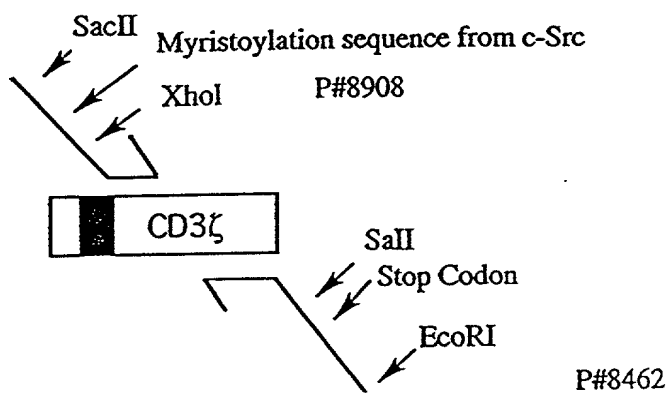


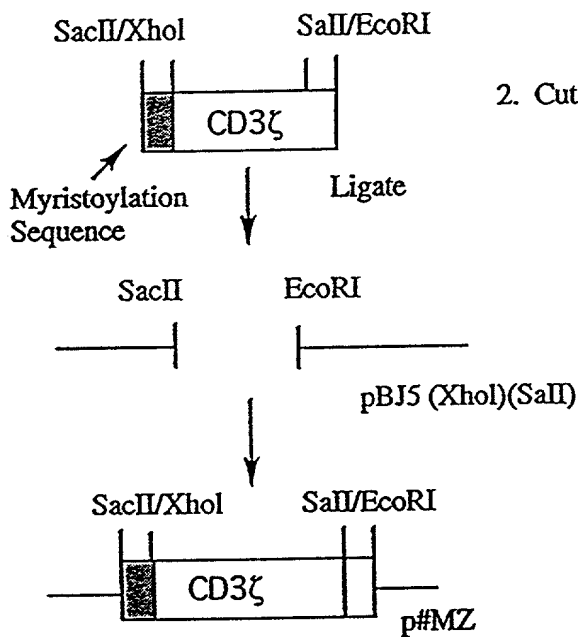
Figure 1/21

Construction of intracellular signalling chimera:

1. PCR myristoylated CD3 ζ



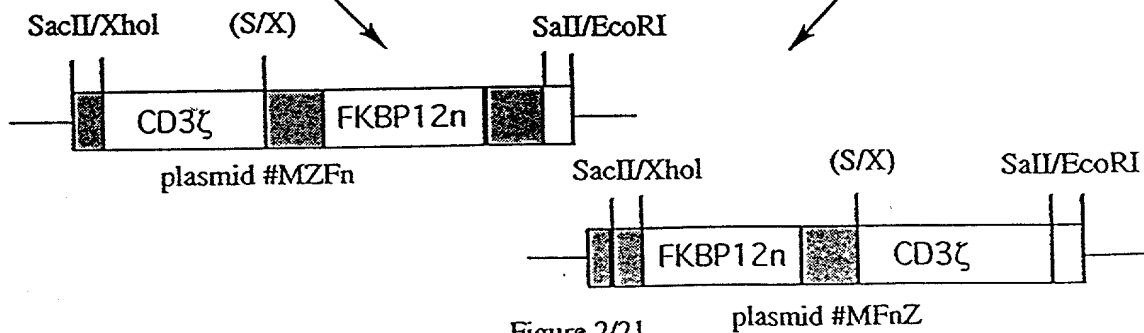
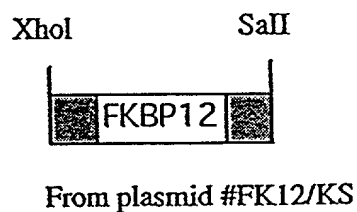
2. Cut and clone PCR fragment



*The MZE series contains a 9aa HA epitope at the 3' end.

3. SEQUENCE insert

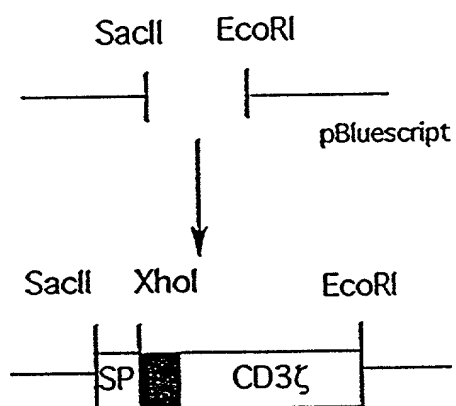
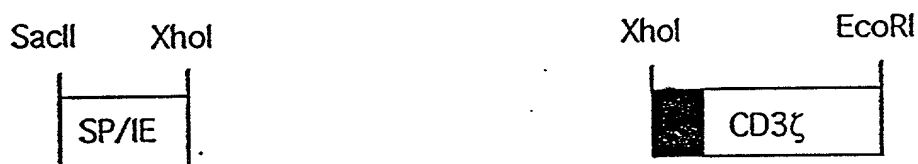
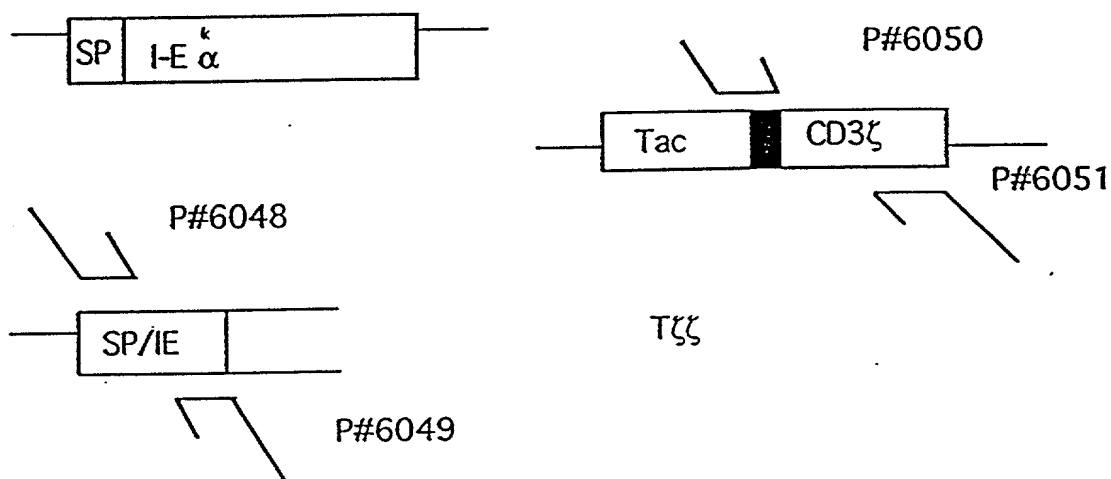
4. Cut at XhoI or SaII and add FKBP domains



Construction of extracellular signaling chimera:

1. PCR murine signal peptide

2. PCR CD3 trans-membrane and cytoplasmic domains



plasmid #SPZ/KS
SEQUENCE insert*

Cut XhoI

10547-1-1304

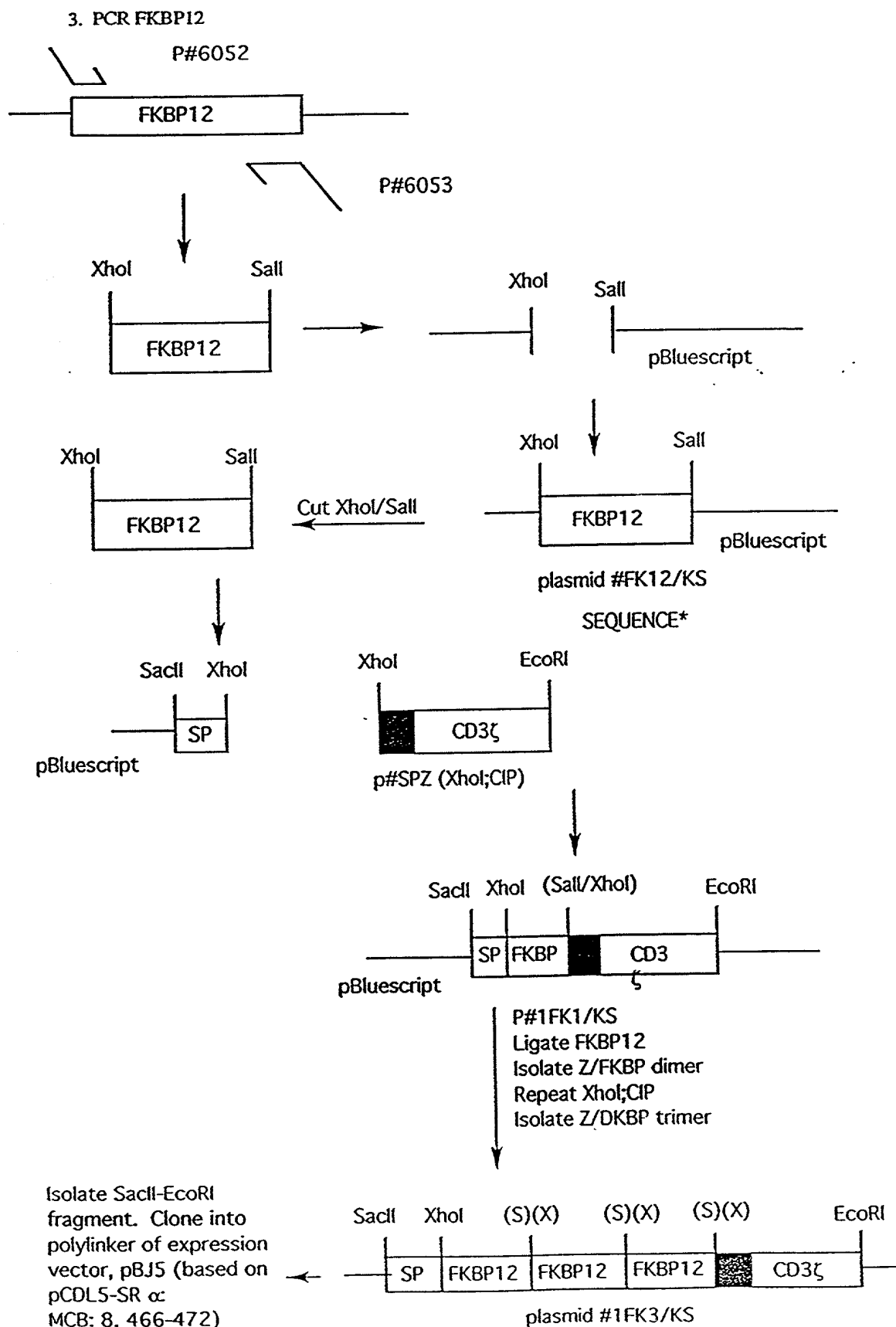


Figure 3B/21

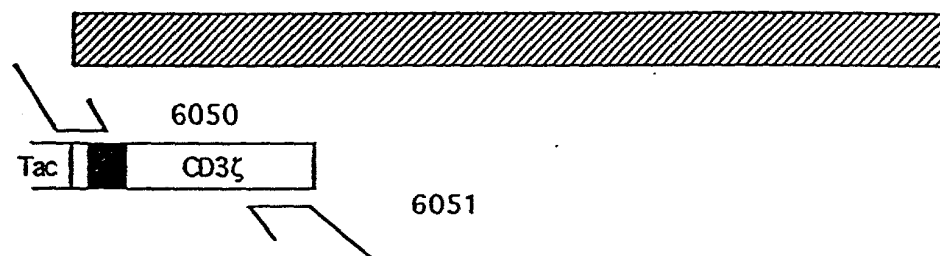
Diagram of a second embodiment of the device. It shows a horizontal rectangular block labeled 6048. Inside this block, the text $IE_{\alpha}^k\text{-SP}$ is written. A line enters from the left and connects to the block. A bracket on the right side of the block is labeled 6049.

6048: 5'-CGACACCGCGGCCACCATGGCCACAATTGGAGC-3'

SacII homology

Kozak M A T I G

6049: 5'-CGACACTCGAGAGCCCATGACTTCTGG
L A W S - 7



Asp-Gyl#1

6050: 5'CGACACTCGAGCTCTGCTACTTGCTAGGTGGAATCCTCTTC-3'

E L C Y L L G G I L F

*A to G

6051: 5' GCGAATTCTTAGCGAGGGGCCAGC-3' *G to C

EcoRI homology

St R P A L

3'Sal #B

8462: 5' GCGAATTCTTAGTCGACGCGAGGGGCCAGGGTC-3'

St R PAL

homology

Cys-Gly #2
 7129: 5' GGGCTCGAGCTCGGCTACTTGCTAG-3'
 L G Y L L

homology
 XhoI *
 *T to G

CYCC

6568: 5'-CGACACTCGAGGTGACGGACAAGGTC-3'

XhoI homology

6569: 5'-CGACAGTCGACCCAATCAGGGACCTC-3'

Sall homology

EPITOPE

7850: 5'-TCGAGTATCCGTACGACGTACCAGACTACGCAG-3'

XhoI BsiWI

Y P Y D V P D Y A

7851: 5'-TCGACTGCGTAGTCTGGTACGTCGTACGGATAC-3'

Sall

EPITOPE: 5SEP, 3XEP

8922: 5'-TCGACTATCCGTACGACGTACCAGACTACGCAC-3'

Sall

8923: 5'-TCGAGTGCGTAGTCTGGTACGTCGTACGGATAG-3'

XhoI

Myristoylation from c-src SSMXZ

8908: 5'-CGACACCGCGGCCACCATGGGGAGTAGCAAGAGCAAGCCT

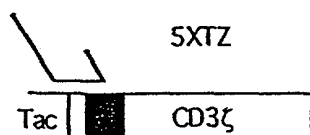
SacII

KOZAK M G S S K S K P

AAGGACCCCAGCCAGCGCCTCGAGAGGAGTGCAGAGACTG-3'

XhoI ζ-homology

K D P S Q R L E R S A E T



8912: 5'-CGACACTCGAGGAGCTCTGTGACGATG-3'

XhoI homology

E L C D D

Figure 4B/21

Asp-Lys #4
 8061: 5'-CGACACTCGAGCTCTGCTACTTGCTAAAGGGAATCCTCTTC-3'
 E L C Y L L K G I L F

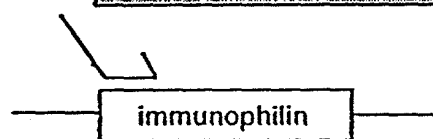
XhoI [homology] **

#4 Extension
 8907: 5'-CGACACTCGAGCTGCTGGATCCGAAGCTCTGCTACTTGCTAAAG-3'
 E L L D P K L C Y L L K

XhoI [homology] *GATtoAAG

TAC-Tm #3
 7220: 5'-CGACACTCGAGACAACAGAGTACCAGGTAGC-3'
 E T T E Y Q V

XhoI [homology]



FKBP12

6052: 5'-CGACACTCGAGGGCGTGCAGGTGGAGAC-3'
 E G V Q V E

XhoI [homology]

6053: 5'-CGACAGTCGACTTCCAGTTT TAGAAGC-3'
 V E L K L L

Sall [homology]



FKBP13
 8460: 5'-TCGACACTCGAGACGGGGGCCGAGGGC-3'
 E T G A E G

XhoI [homology]

8461: 5'-CCGACAGTCGACCTCTATTTT GAGCAGC-3'
 V E I

Sall [homology]



Figure 4C/21

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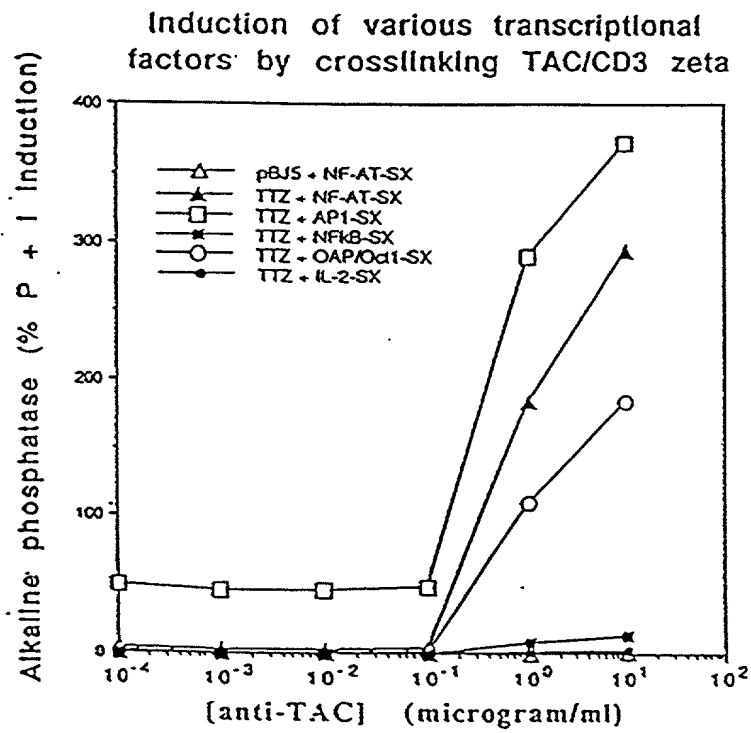


Figure 5/21

Inhibitory activity of dimeric FK506 and CSA

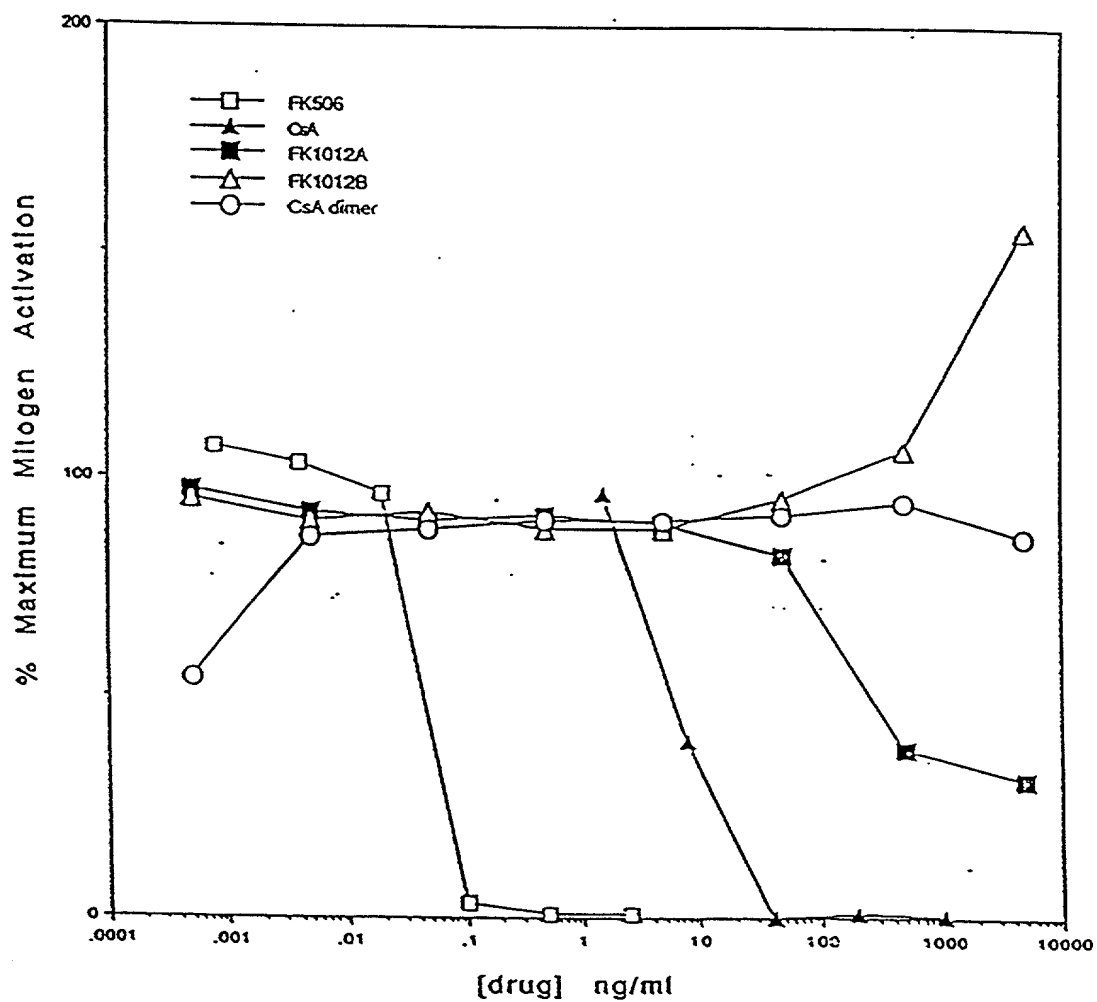
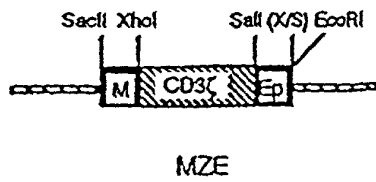
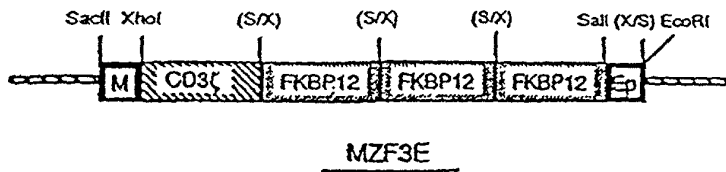
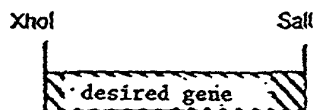
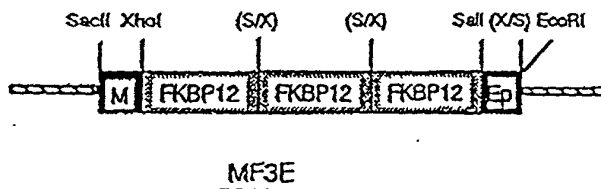


Figure 6A/21



Cut XhoI/SalI; CIP; + FKBP12 X 3



1. Cytoplasmic moiety of surface receptor
2. Tyrosine Kinase
3. Transcription Factor
4. Others

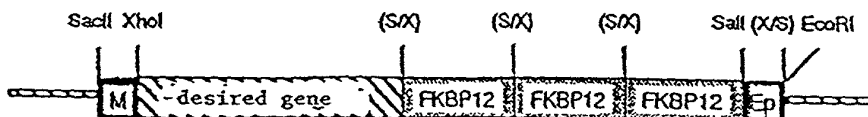


Figure 6B/21

Activity of FK1012A on the chimeric FKBPX3/CD3 zeta receptor

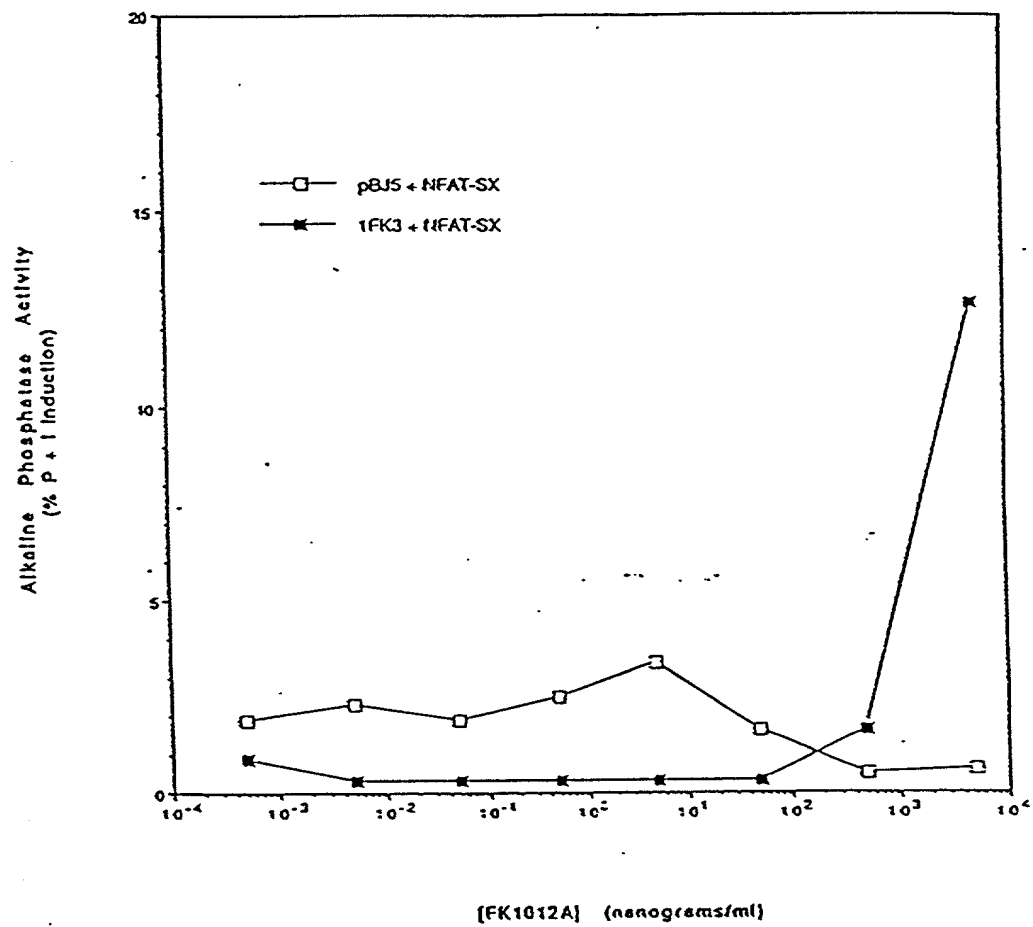


Figure 7/21

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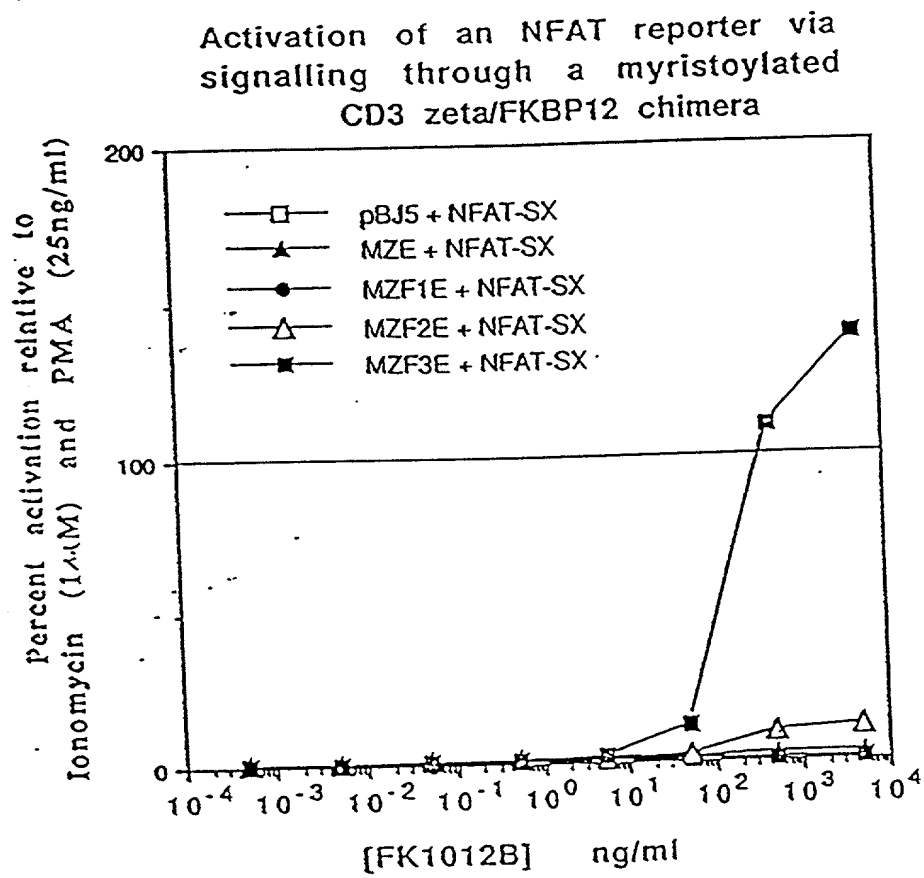


Figure 8/21

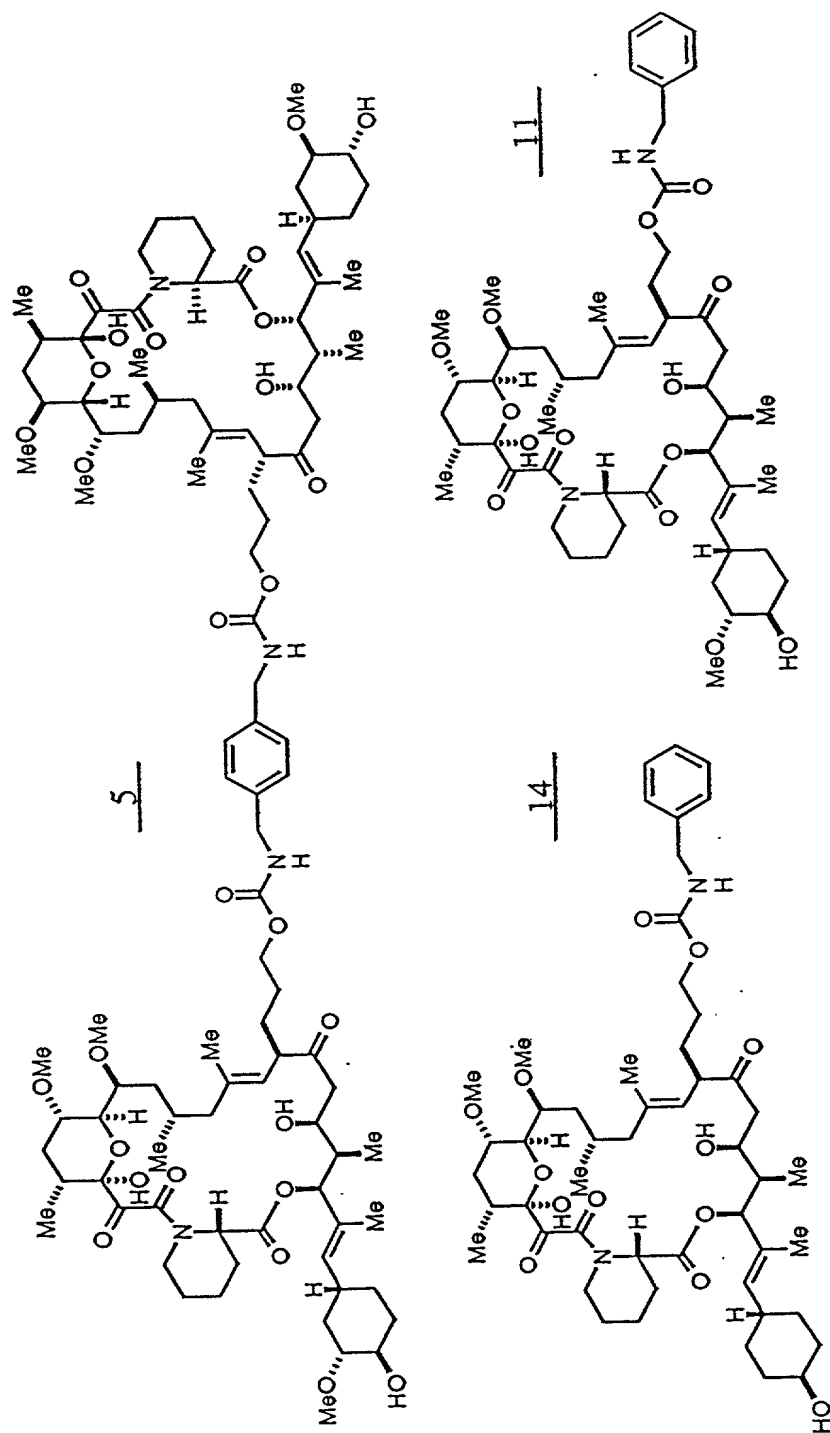


Figure 9A (#1)/21

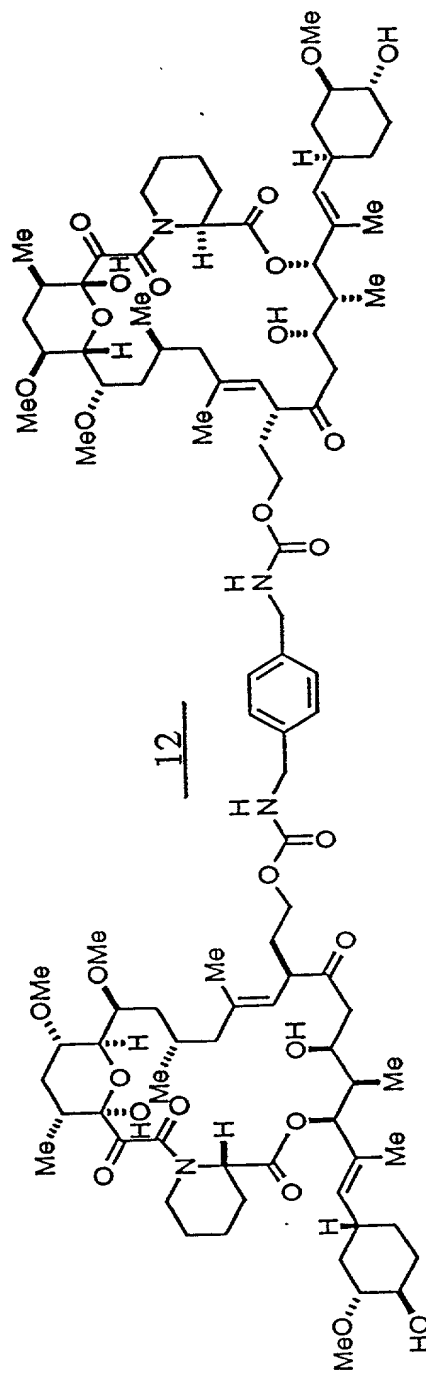
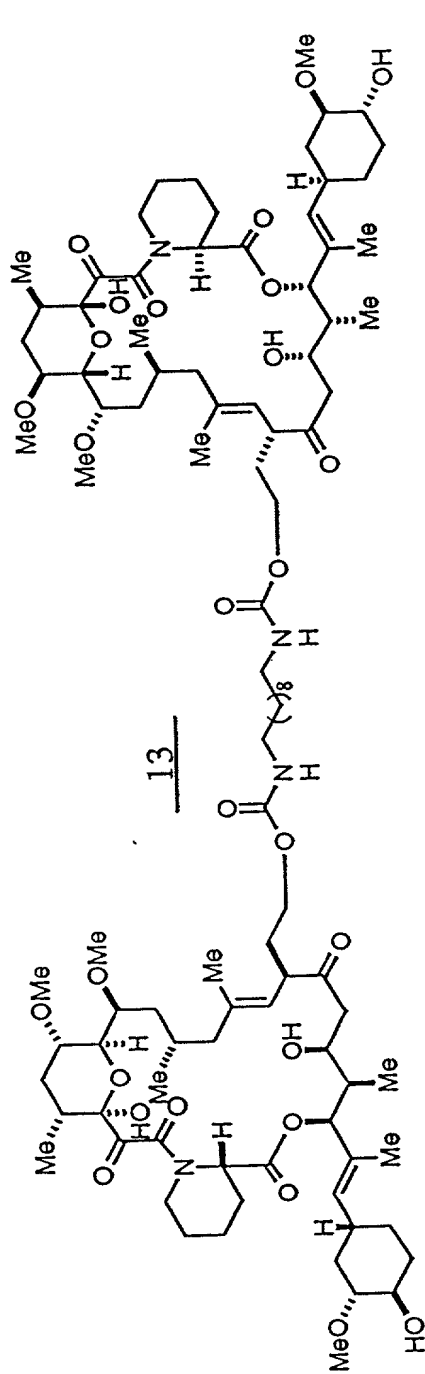


Figure 9A(#2)/21

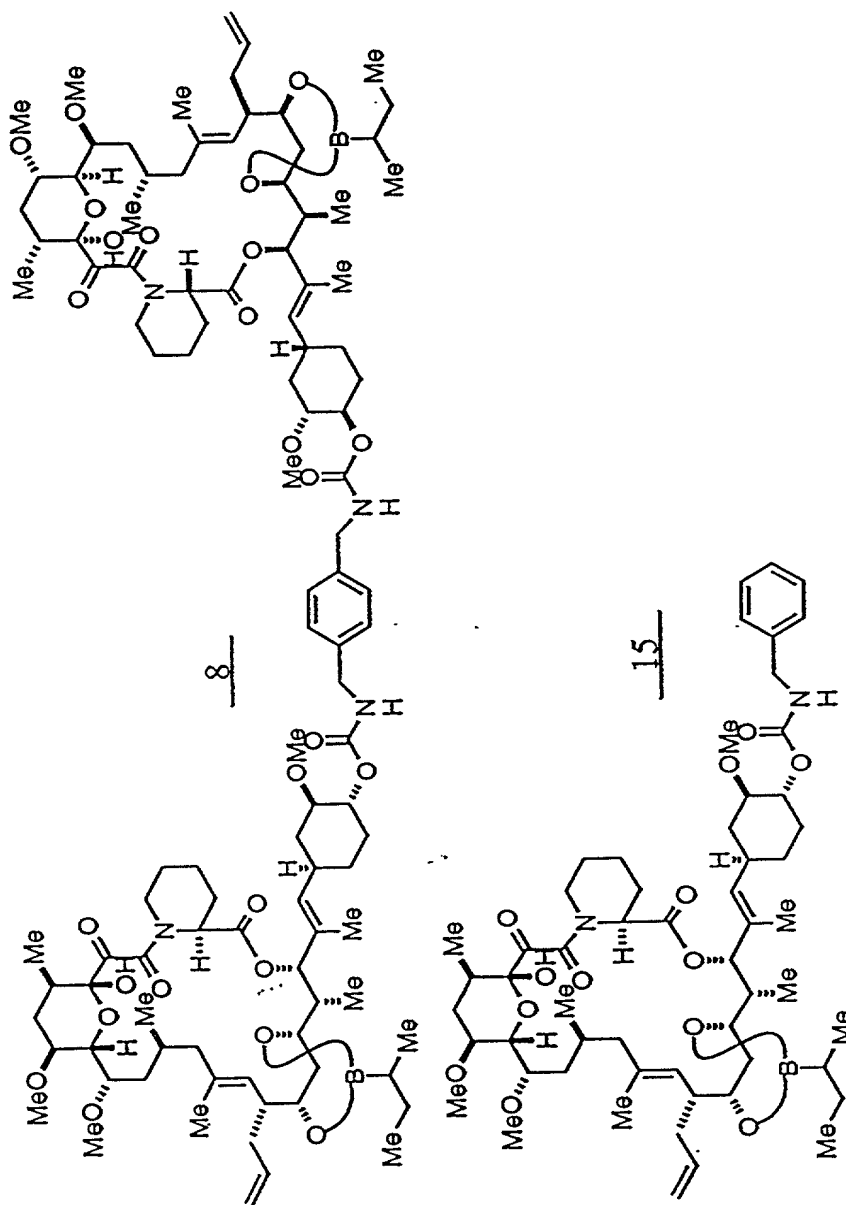


Figure 9B (#1)/21

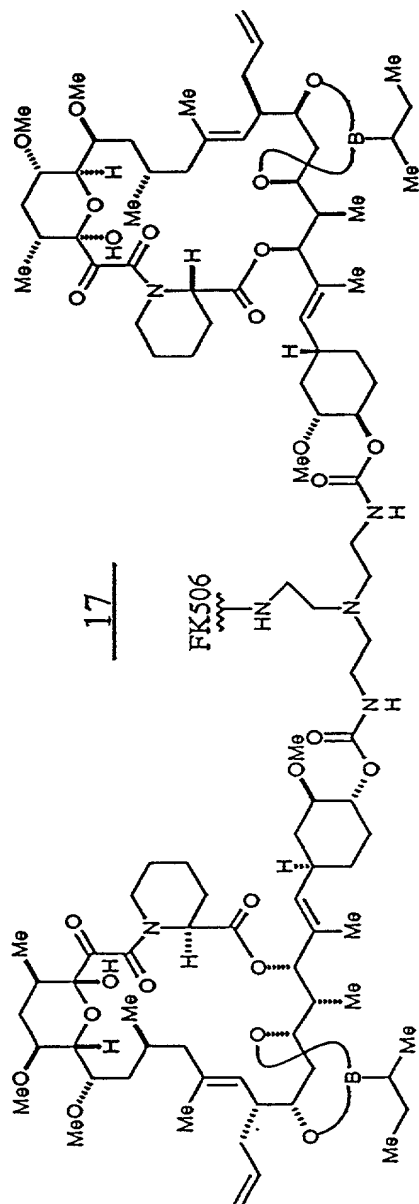
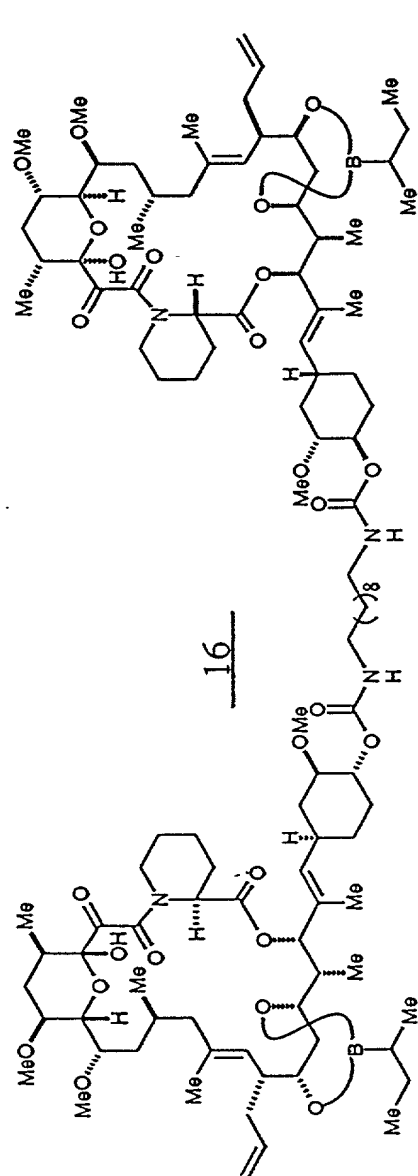


Figure 9B (#2)/21

Scheme 1

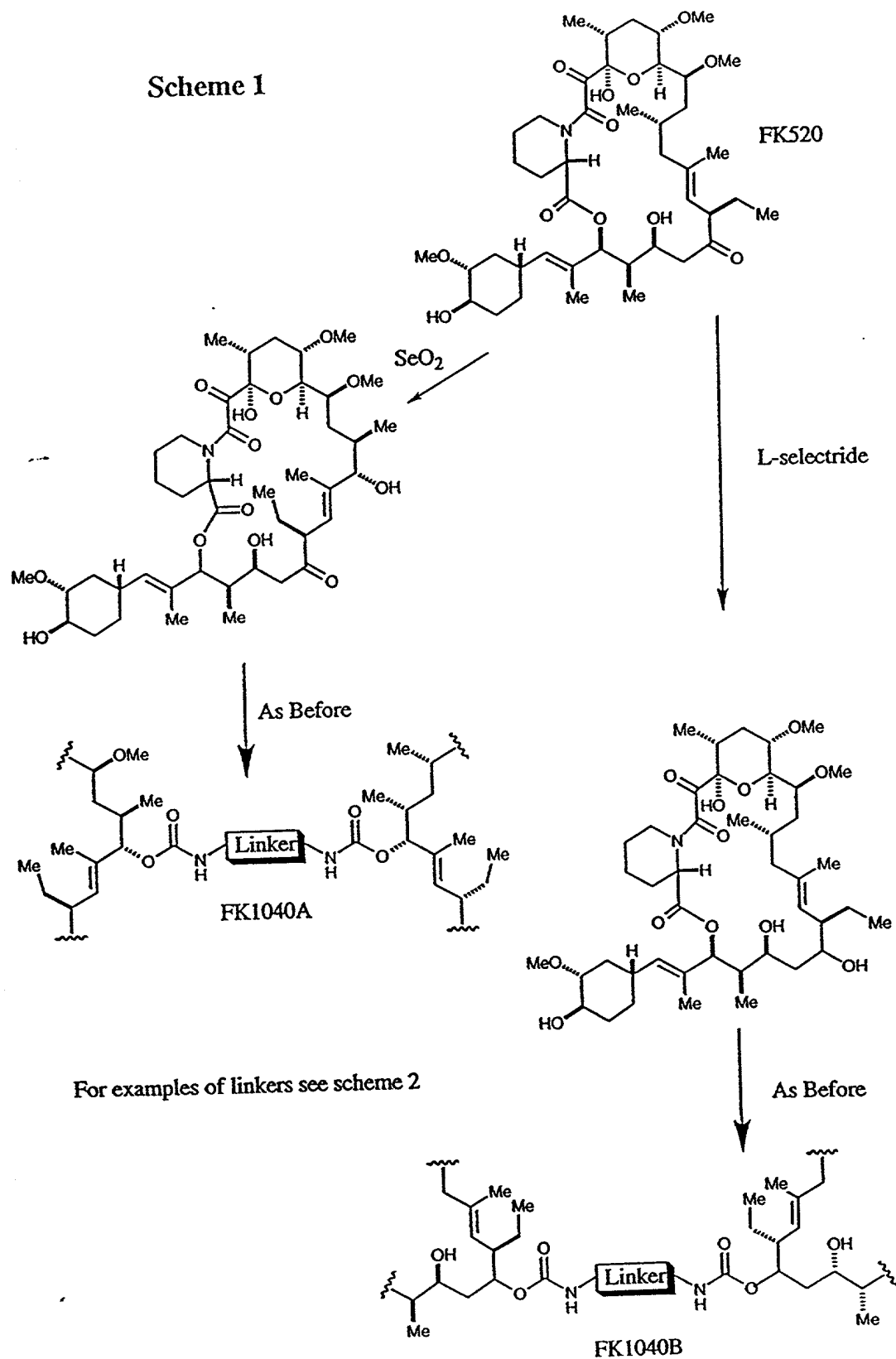


Figure 10/21

Scheme 2: Synthesis of Dimers

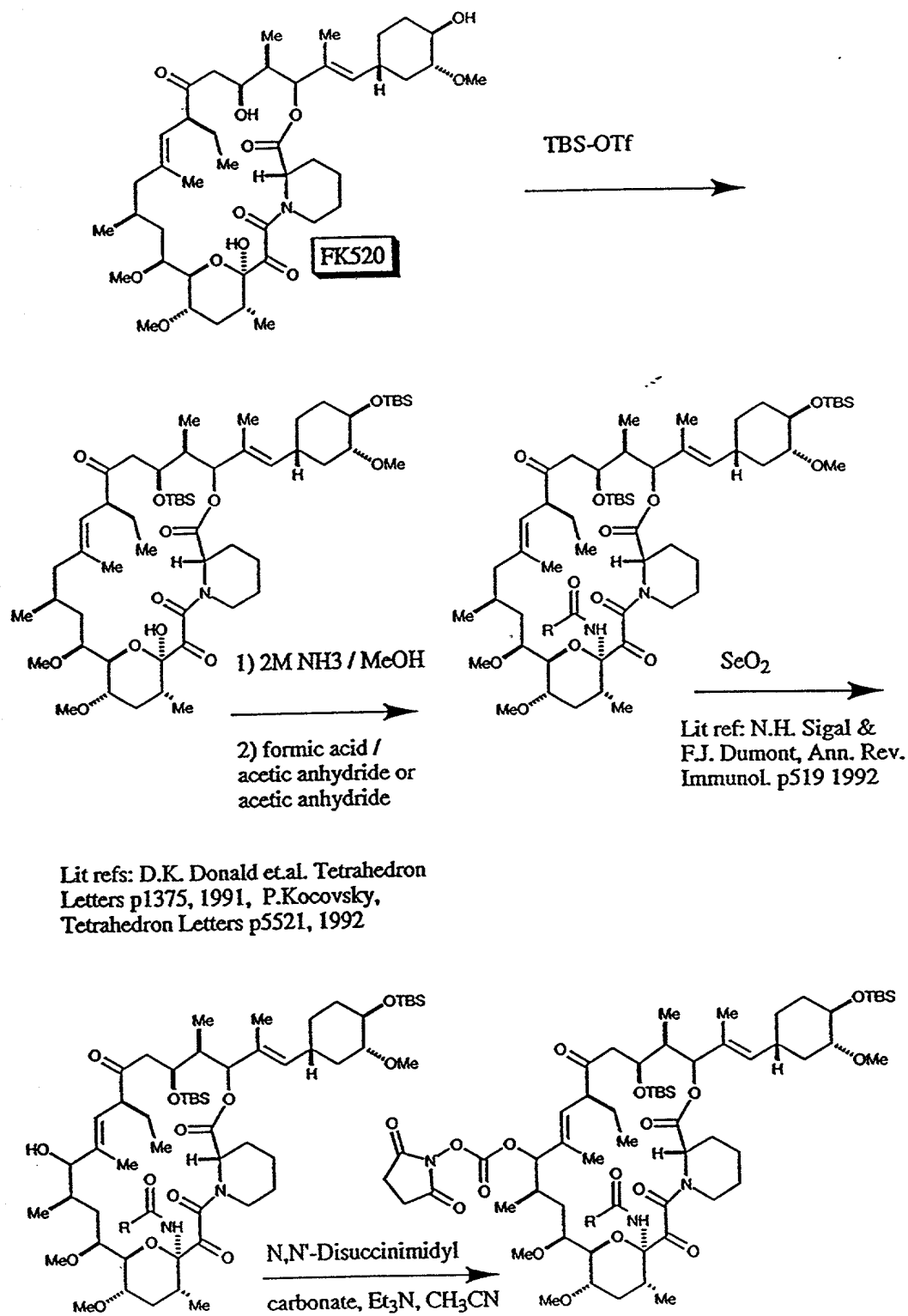


Figure 11A/21

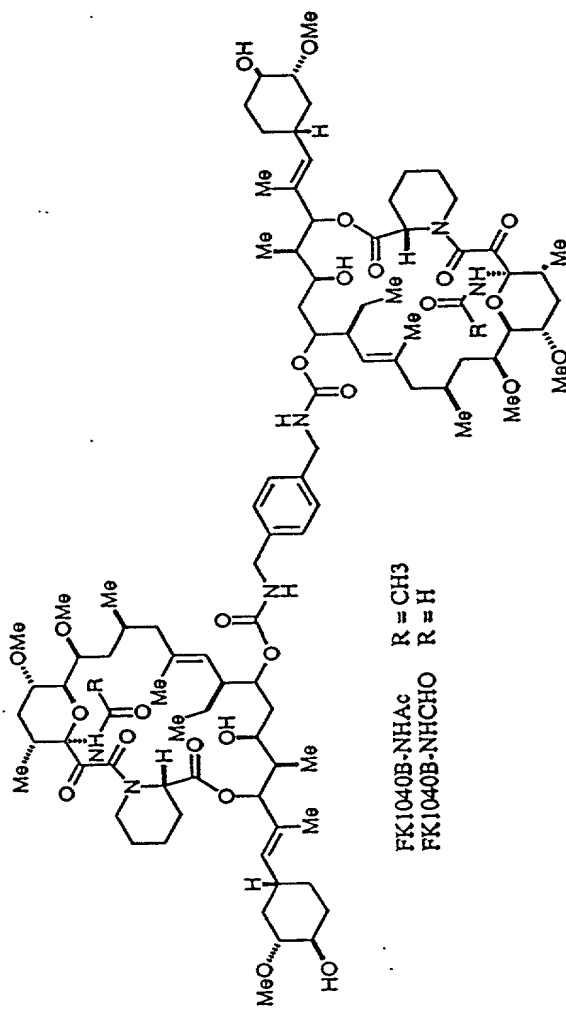
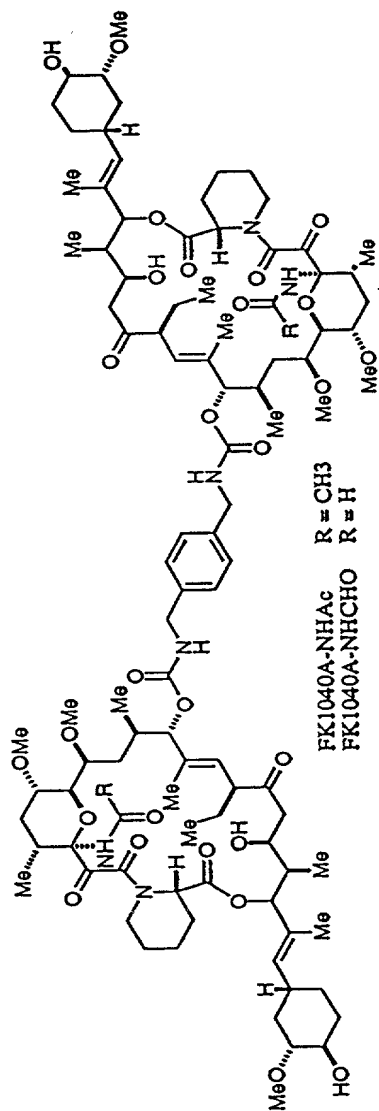


Figure 11B (#1)/21

An additional modified FK520 (FK1040) that interferes with FKBP12 yet should bind the FKBP12 mutant: F36A or F99A or Y26A, or combinations thereof is

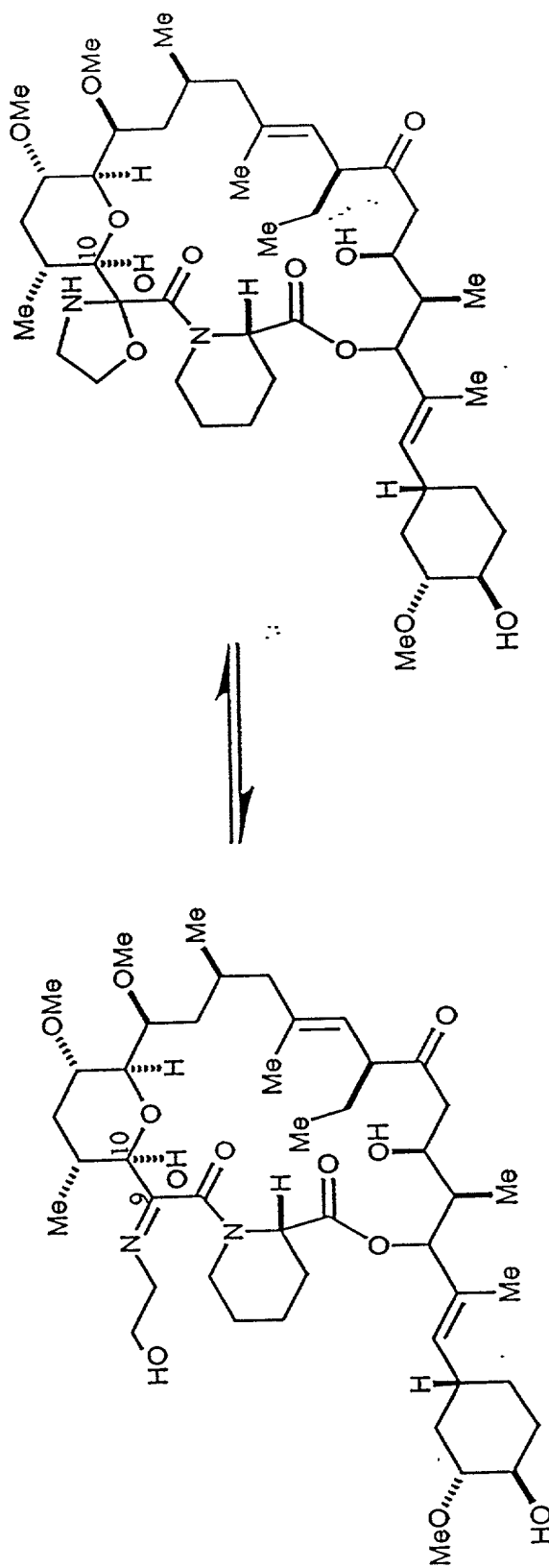


Figure 11B (#2)/21

Scheme 3 Heterodimerization

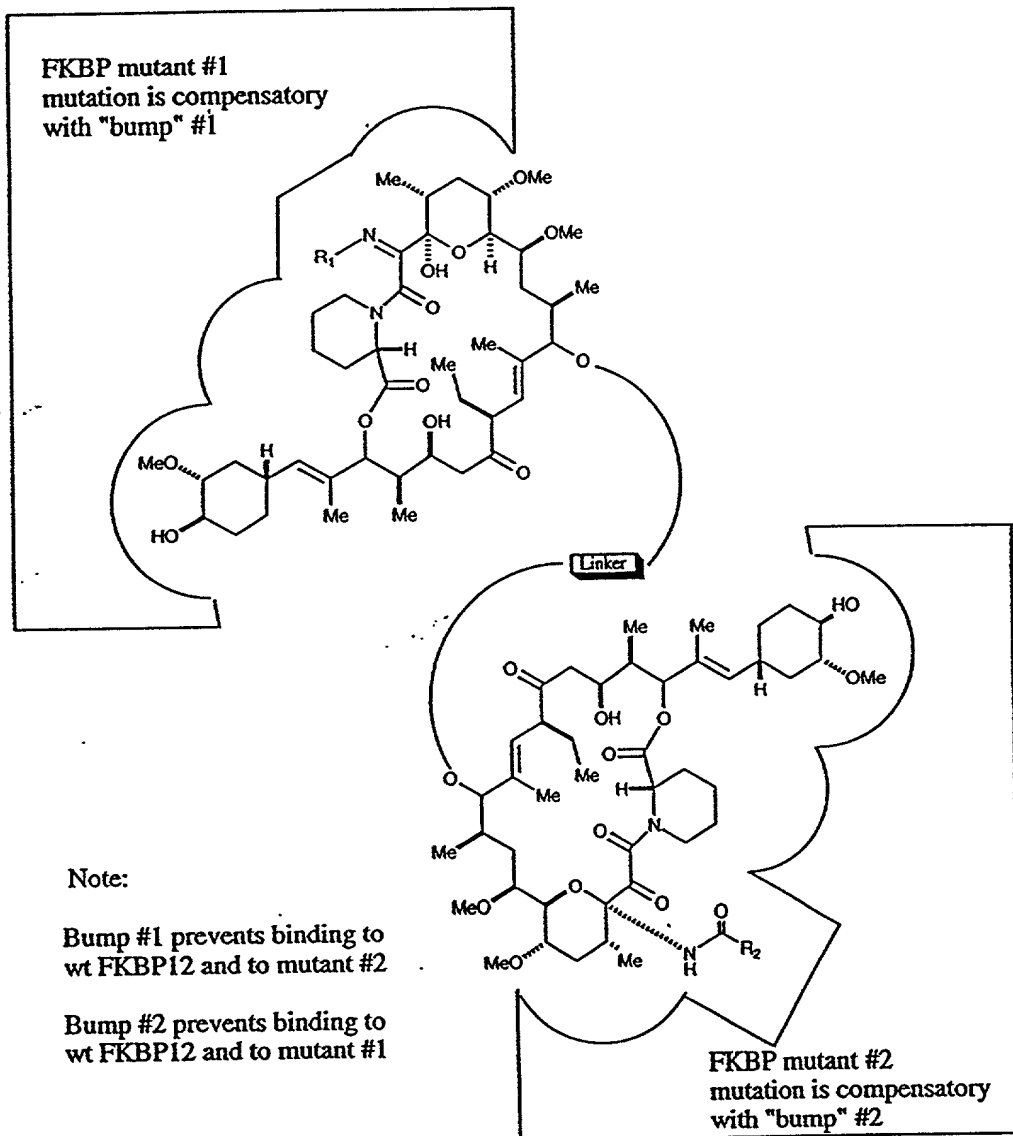
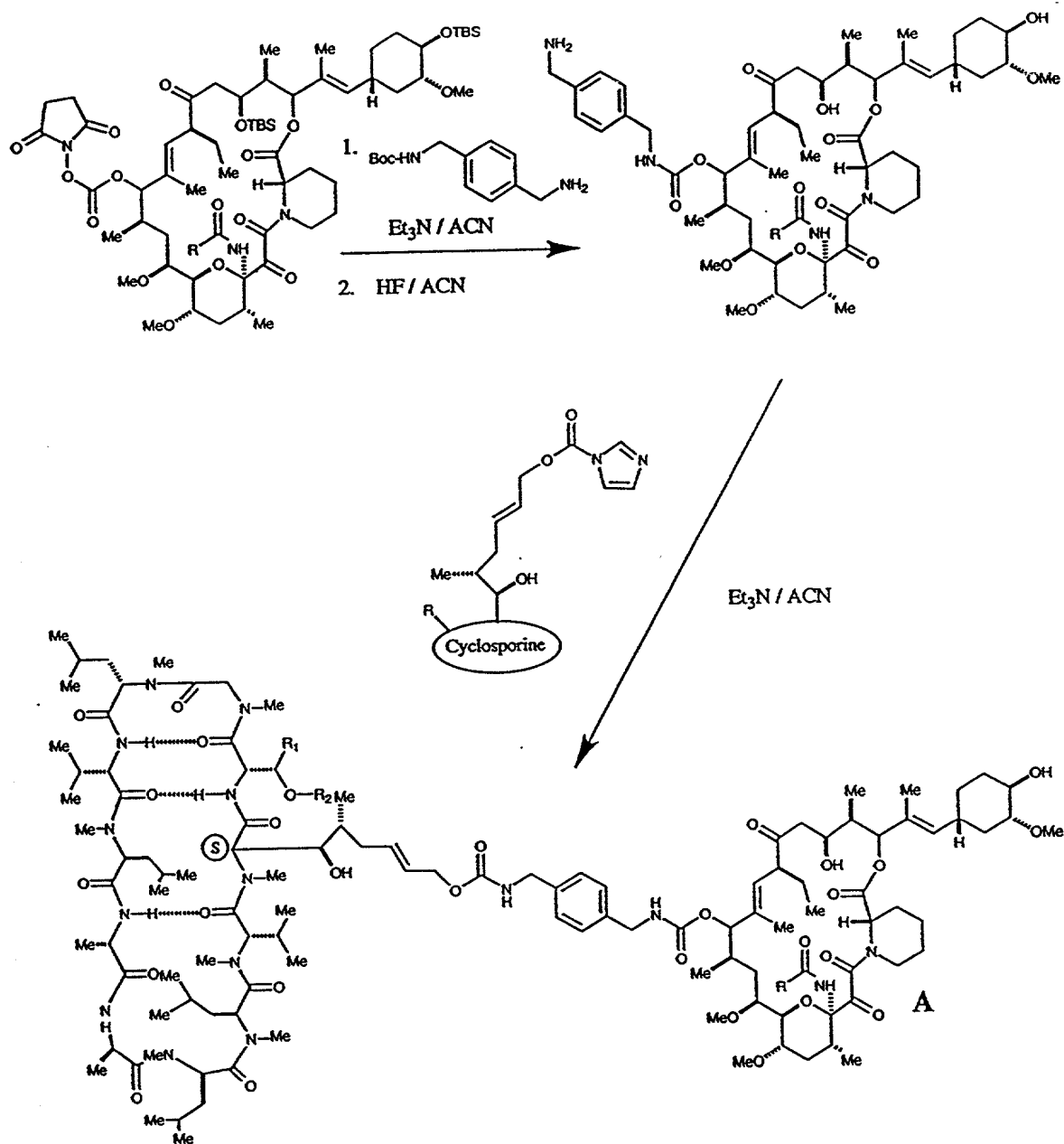


Figure 12/21

Scheme 3: Synthesis of heterodimers



In this example, a heterodimer of a cyclosporine analog and FK520A-NHCO-R were heterodimerized. However, the scheme can easily incorporate other FK506/520 derivatives to form hetero or homodimers

Figure 13/21

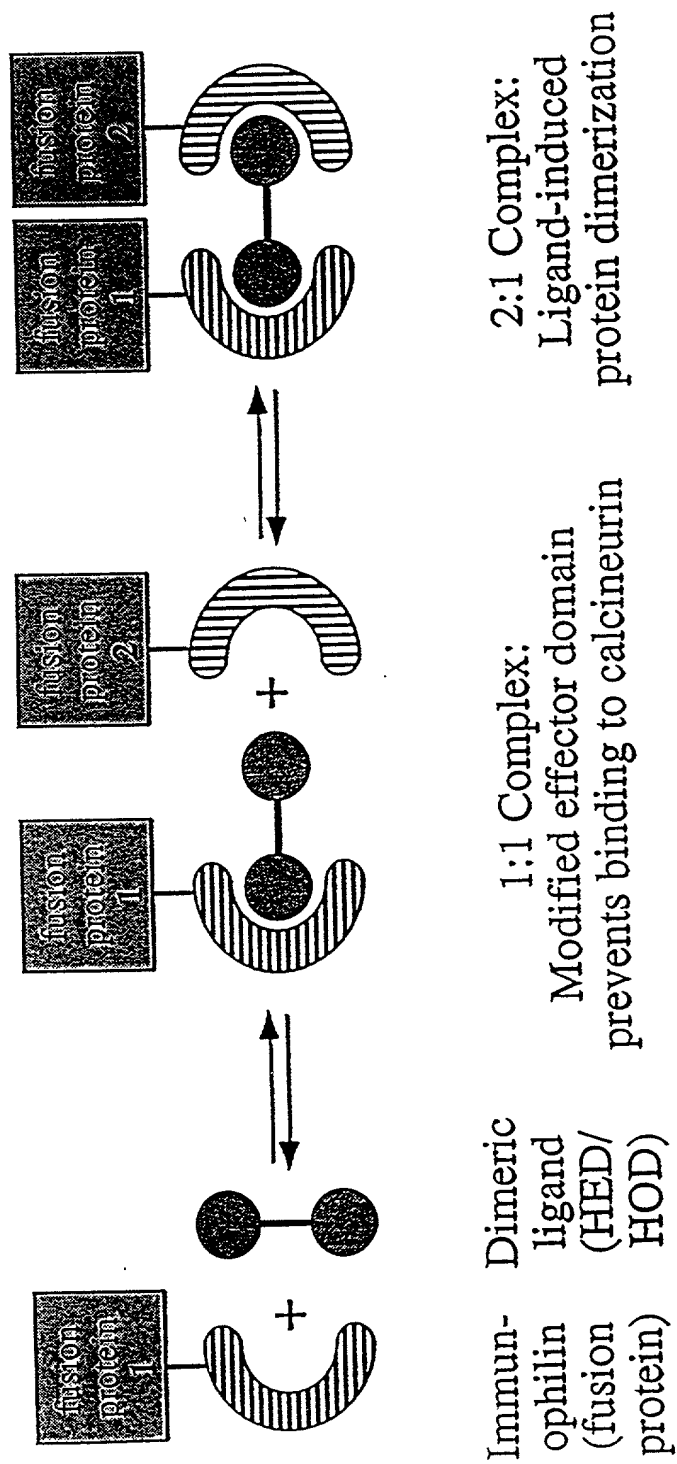


Figure 14/21

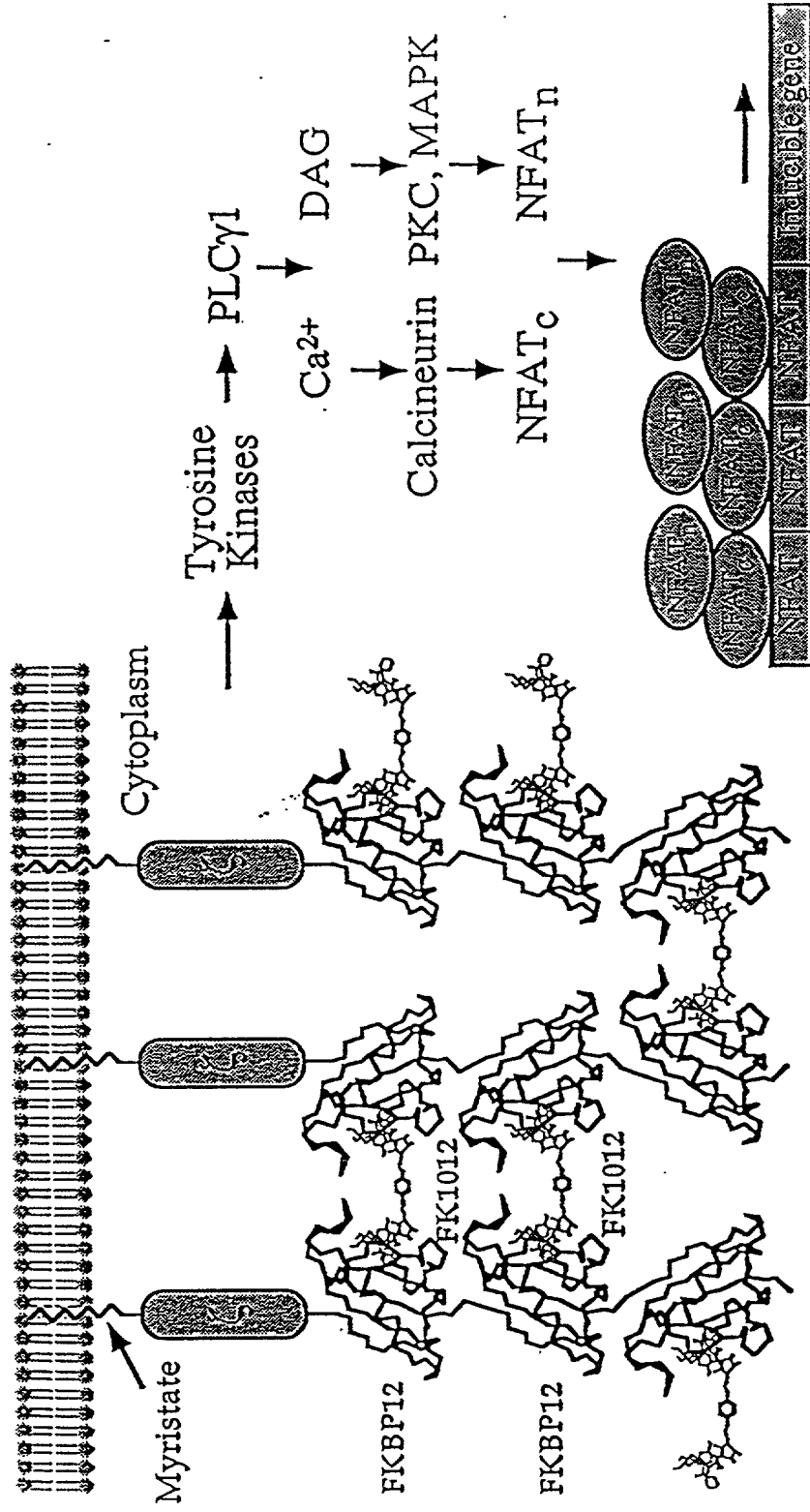


Figure 15/21

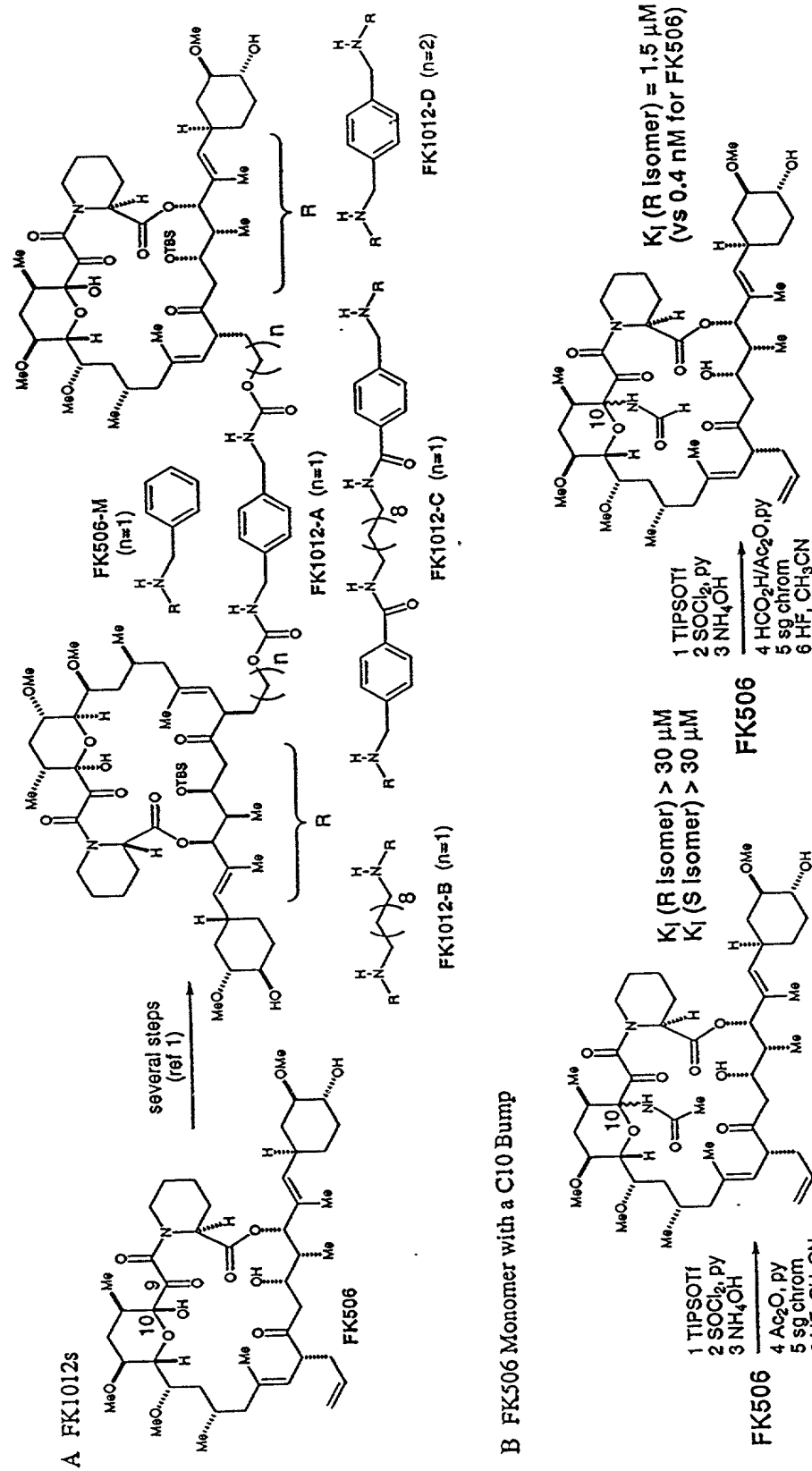
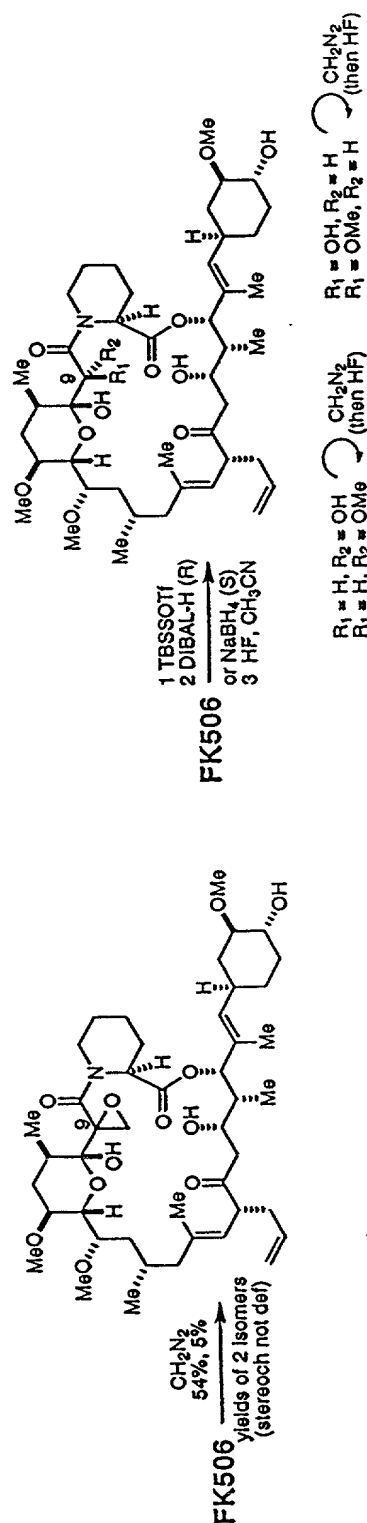


Figure 16 (#1)/21

C FK506 Monomer with a C9 Bump



D HED Reagent Synthesis

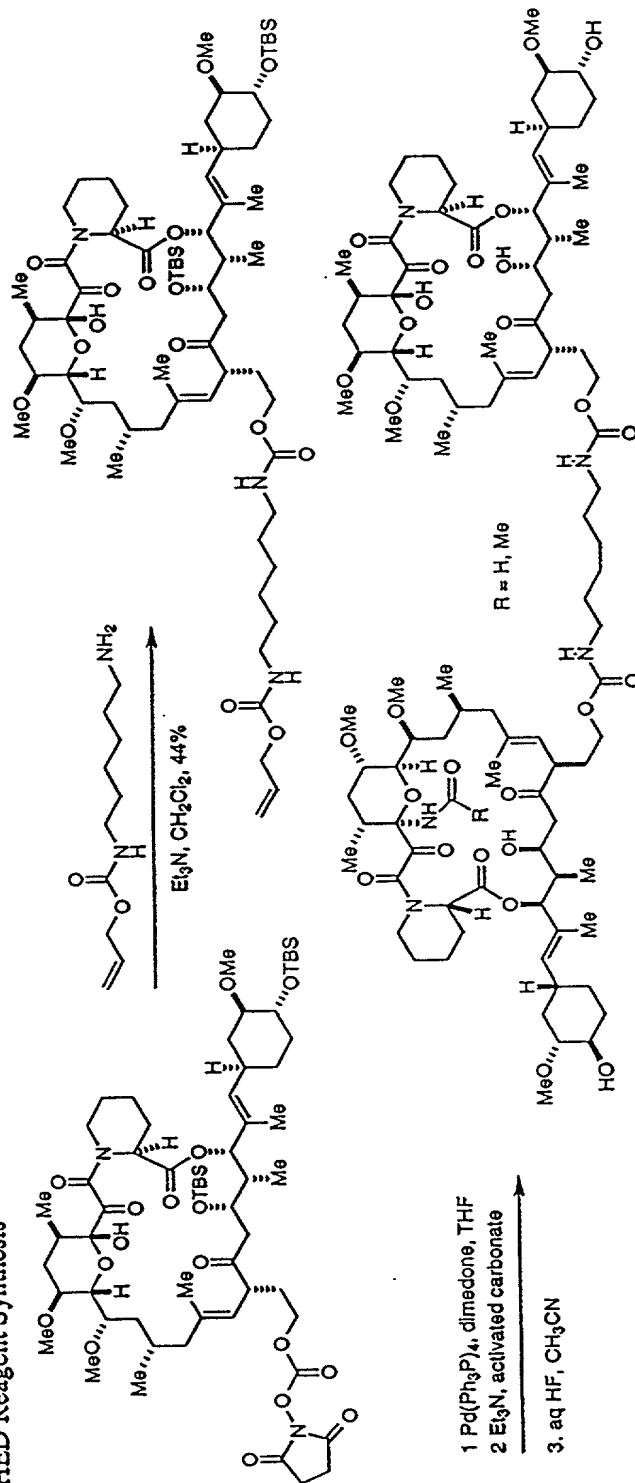


Figure 16 (#2)/21

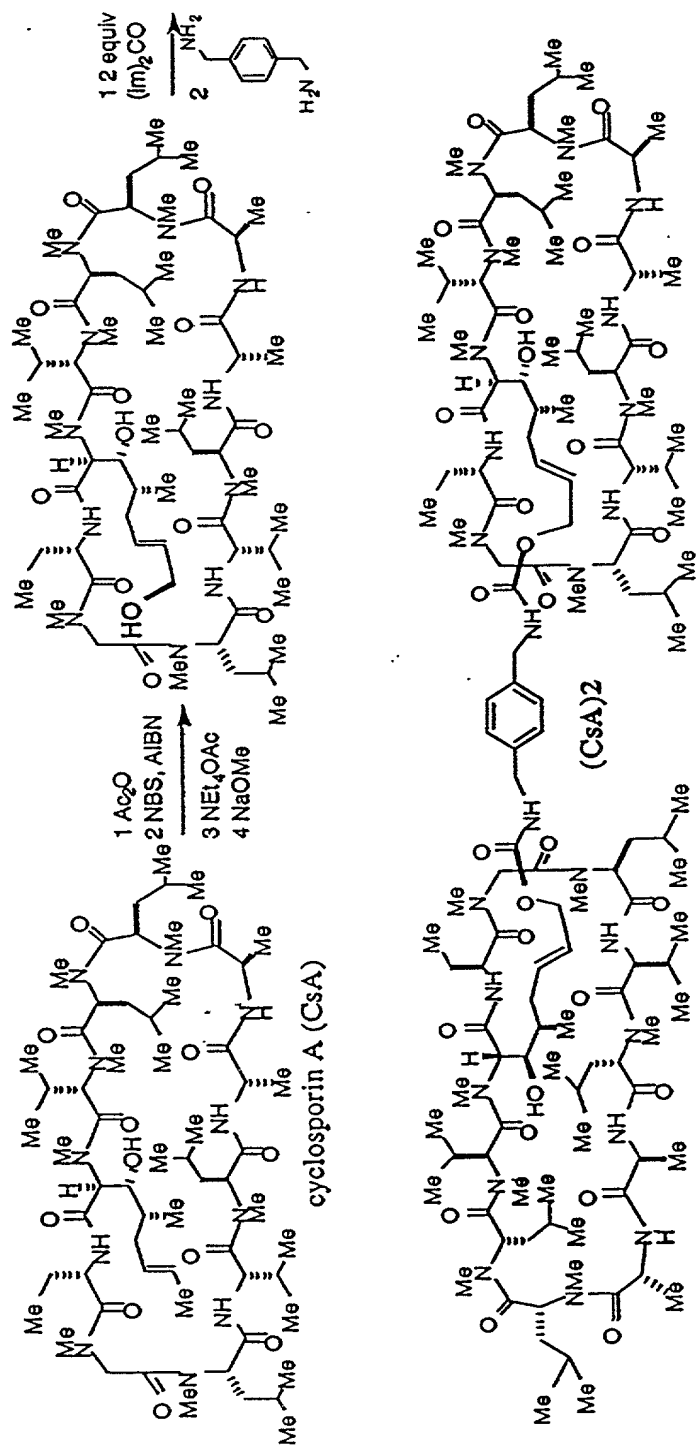


Figure 17/21

A cDNA construct

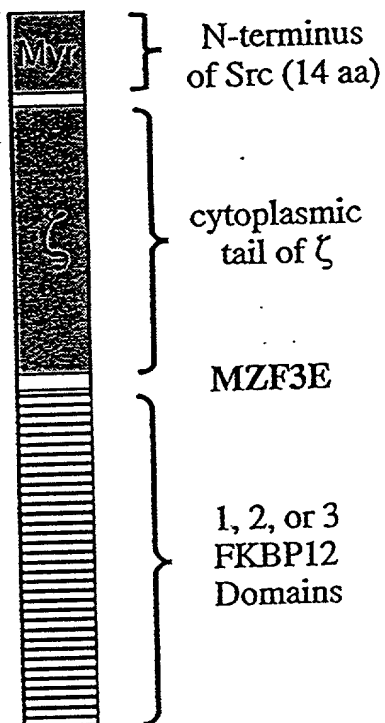


Figure 18A/21

B expressed protein

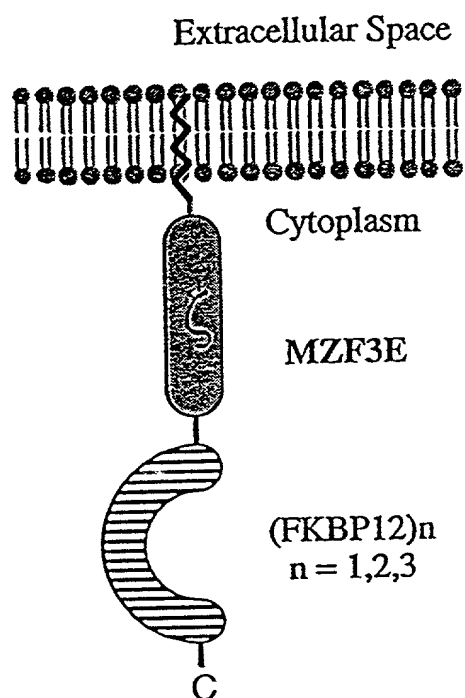


Figure 18B/21

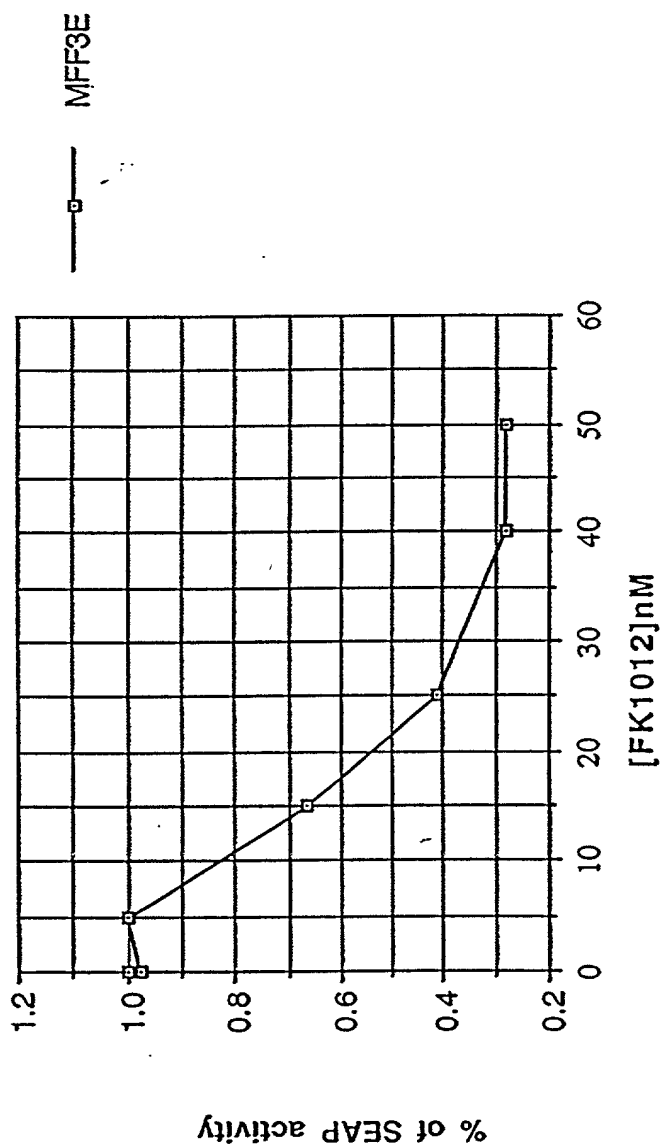


Figure 19/21

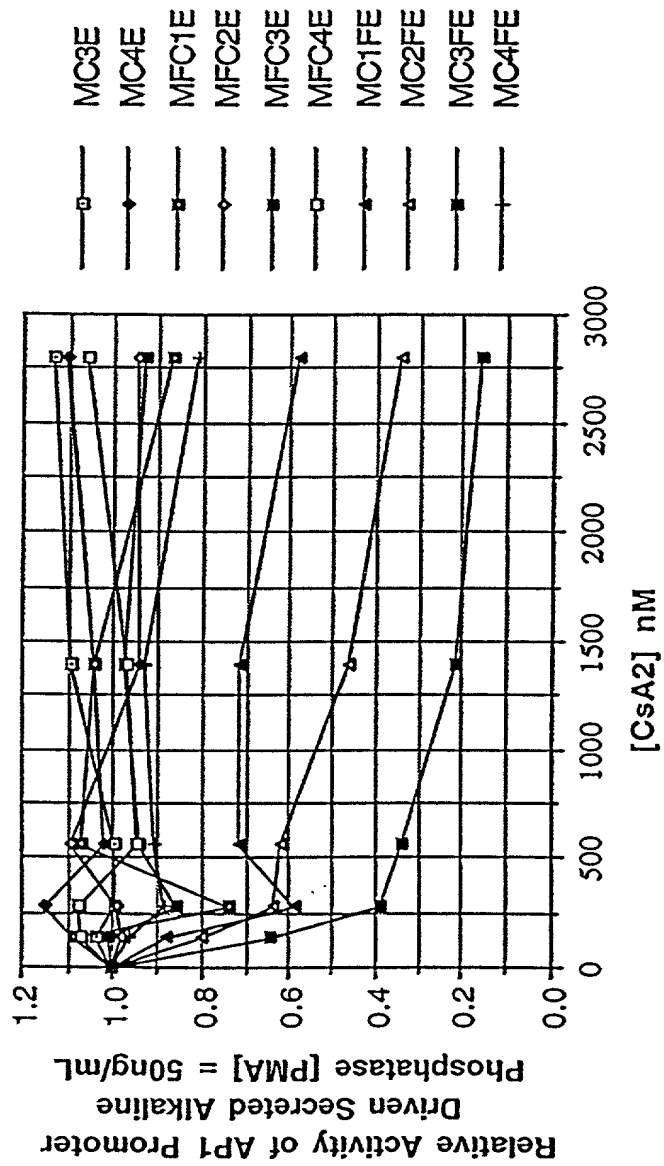


Figure 20A/21

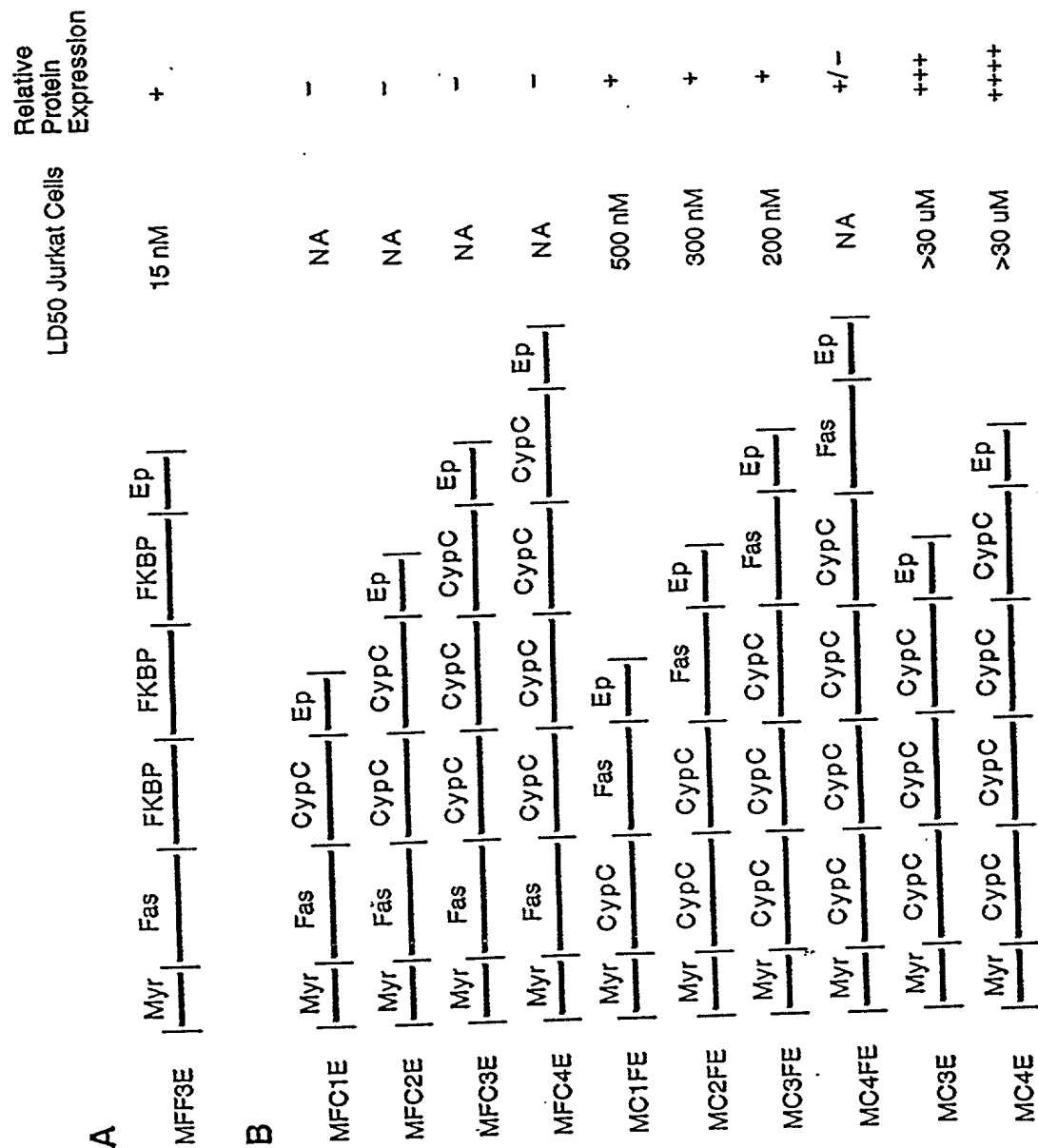


Figure 20B/21

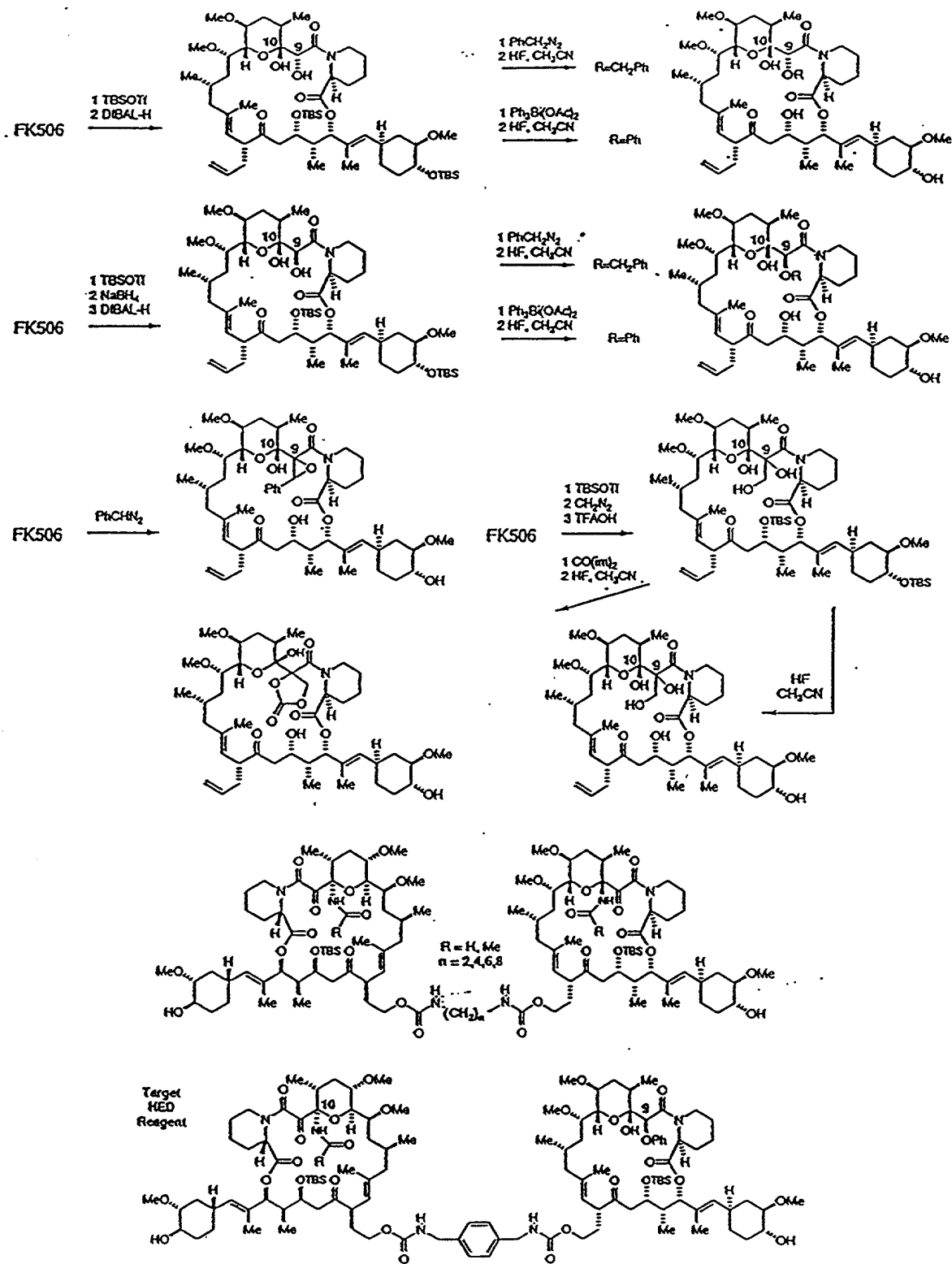


Figure 21/21